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Innovative Approaches to Breeding Cotton

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Indian cotton yields have been stagnant at 437 to 566 Kg/hectares (ha) from 2004 to 2018. The 15-years (2004 to 2018) average yield (511 Kg/ha) of Indian cotton is 44% less than the 15-years average yield (905 Kg/ha) of rest of the world (Kranthi, ICAC, personal communication). Cotton in north India is grown under almost completely irrigated conditions on about 1.5 million ha. Even the 15-year average yield of 567 Kg/ha in irrigated north India is 37% less than the 15-year average yield of rest of the world, where only 45% of cotton is grown under irrigation (Kranthi, ICAC, personal communication). Indian cotton yield ranks below at least 32 countries. The low rank is despite hybrid technology, double-gene-Bt, high fertiliser use, 35% irrigated area, access to pesticides and other technologies — advantages that many African countries do not have, yet they are ahead of India in yields. This paper explores innovative approaches to breeding cotton that can catalyse high yields in India with lowered production costs.

Cotton is a unique commercial crop, which constantly encounters an array of intimidating problems that bring down the remunerative value of the crop. There are problems of biotic stresses like bollworms, sucking pests and viruses, which constantly evolve and adapt to break down the barriers of host-plant resistance. Agriculture, especially cotton cultivation in the Indian subcontinent, still continues to be labour intensive and least mechanised. The current bushy type of plant architecture in India is not well suited for mechanical planting and harvesting. Further, the planting density in India is low and the crop season extends over 5-8 months, which requires more labour for crop production and multiple pickings. The increasing labour costs in cotton cultivation result in declining returns. It has now become necessary to explore options to alter the plant type to make it as compact as possible so as to increase the planting density, condense the growing season to 5 months and make it amenable for mechanised planting and harvesting. Compact cottons have the primary advantage of planting in high density thereby contributing to high yields coupled with mechanised cotton cultivation, reduced intensity of pest damage due to a shorter season and facilitating a double-cropping system for ecological sustainability and enhanced remuneration in cotton-based cropping systems.

Interestingly, even the Indian seed industry has been contemplating a change in its mindset to develop short-season Bt hybrids with compact architecture due to their ability to escape the late season pink bollworm infestation and their suitability for machine sowing and picking. However, there is a practical limit up to which compactness can be introduced in hybrids to reduce spacing and increase density, as it leads to increase in hybrid seed cost. In contrast, it is possible to introduce a high degree of compactness in varietal background to easily increase the density to more than 100,000 plants per hectare. It is thus possible to reap the benefit of varietal compactness to a much greater extent than compactness in hybrids. It is necessary for public sector institutions to focus research on developing gradations of compact types and determine the ideotype of cotton for different growing conditions in India. There is a need for research work to determine the optimum height of the productive compact cotton varieties grown for manual picking at present and for machine picking to be followed in future under different situations. Presently, cotton is manually picked in the Indian subcontinent and a plant height of 6-8 feet is common. For machine picking, the plant dimensions are restricted to 3 feet high and 2 feet wide so that the plants do not break when they pass through the spindle assembly window. Thus, the future plant types being developed for high density planting should be suitable for machine sowing and there should be a scientific debate among breeders on the ideal height for manual picking and whether tall compact plant types can out-yield dwarf compact types under different crop production systems in India. In any case, the plant type should be accessible for weeding, spraying with tractor and such other forms of mechanised operations. Importantly, the cost of varietal seed in high density is much lower compared to hybrids apart from making farmers self-sufficient for their seed needs.

There are several challenges with breeding for compact plant types. While it has taken several years for countries such as Australia, Brazil, China, Mexico, Turkey, Uzbekistan and USA to develop varieties with compact architecture and premium quality fibres, efforts in India are in their infancy. Modernisation of textile industrial operations constantly raises the benchmark of fibre quality requirements. When breeders in India opt for developing compact types with earliness, they still wish the plant to retain a larger boll size for convenience of manual picking, coupled with high fibre quality and synchronous maturity. However, this involves a contradictory combination of traits which goes against the general trend of correlations existing among them. When requirements of genetic improvement become so complex, the approaches of breeding should also match the challenge to become advanced and comprehensive.

A thorough understanding of principles of population genetics and quantitative genetics is fundamental for planning and execution of breeding approaches for creation and exploitation of genetic variability for higher genetic gains. Every breeder, even in remote research centres, addresses these complex issues in his own way and there is no system available for assessment of magnitude of useful variability released and the extent of genetic improvement achieved in the attempts. There should be a simple reporting system among breeders that incorporates the results of successes, reports of failures. assessment of reasons of failure, mistakes committed and their impact in terms of reduced genetic improvement. These studies should act as guides to other breeders in pursuing different breeding approaches in cotton and even other crops. This will help breeders learn and benefit from prior breeding attempts.

The harvesters currently used in developed countries demand the choice of compact cotton genotypes ideally suited for such machine picking. The kind of pickers chosen for use will have a bearing on the plant type suiting the situation of cotton cultivation. While research on developing plant types suited for machine pickers continues, research should also be contemplated to modify tractor drawn cotton picking machines to suit the small-medium holder conditions of India and also to develop back-pack type of pickers for the resource-poor small-holder farmers.

Genetic improvement of cotton is aimed at developing improved varieties and potential hybrids for which a sound knowledge of principles of plant breeding and genetics is fundamental to guide the various stages of genetic improvement. Lack of this leads to oversimplified procedures and defective execution of steps at different phases of varietal improvement. Due to this, though the amount of time and energy involved in the procedures for creation of variability and its exploitation remains same, realised genetic gains produced by breeders remains low. This paper dwells on explaining the genetic basis in deriving modifications in conventional breeding methods. It shows how it is necessary to extend some principal steps of breeding cross-pollinated (random mating system) crops and blending them with routine procedures of self-pollinated crops to improve the efficiency in genetic improvement of cotton for both varietal and hybrid development. Whenever possible, outcomes of our own research findings are summarised and presented in support of the concepts and in some areas, modifications in procedures/steps are suggested based only on explanation of genetic basis of the innovative approaches and modifications in steps of cotton breeding.

Genetic complementation of parents for creation of useful variability

Success of varietal improvement depends up on the magnitude of useful variability availed for a large number of traits such as yield, fibre quality and stress tolerance, followed by efficient exploitation through selection. Desirable variability for an array of these traits will be released only when the parents chosen for hybridisation are genetically diverse enough to complement for all these traits and as a result the F_1 progeny segregates for this large number of loci.

National programs for creation of pool of desirable alleles and structuring transgressive segregants blending desirable traits

Success of a national programme depends on creation of sufficient positive variability and successful exploitation of the same to derive desired potential transgressive segregants. If a single attempt of genetic improvement has to address such complex group of traits and needs of cotton plant, attempts of hybridisation for varietal improvement should systematically address complementation of the parents for the entire range of these traits. Generally, it is very difficult to get two parents that are perfectly complementary for the entire range of traits. It is necessary to choose diverse sets of parents complementing each other for a range of traits of interest and utilise them scientifically to create a wide range of variability through innovative breeder approaches and novel methods of genetic improvements. The distribution of desirable traits in a parent must determine its contribution to the pool of favourable alleles generated. Thus, the contribution of each parent to the pool of alleles could be same or different. In the development process, the expertise of entomologists, physiologists and agronomists can be crucial in determining the score card of each genotype in terms of desirable expression for traits of economic importance like yield, its trait components, fibre quality, stress tolerance, biomass features, harvest index and relative growth rate.

When such an integrated attempt is made, it is possible that variability is created for a very large number of loci with huge variability for isolation of potential transgressive segregants blending a big list of desired traits. The huge magnitude of variability released by the large sets of parents cannot be managed by individual breeders. Therefore, concerted efforts should be made regularly through national programs to create enormous positive variability by supporting a large group of breeders identified in a region who would screen the large populations and distribute them for practicing selection and exploiting the same for genetic improvement.

Choice of a set of parents for hybridisation can be effectively done by assessing the score card of genotypes for an array of requisite traits to decide upon the group of parents that can complement each other for the entire range of traits. It is difficult to expect just two parents to perfectly complement each other for the entire range of yield components either influencing biomass or translocation of photosynthates to sink, (as shown in Fig. 1) biotic, abiotic stress related traits and an array of other desired traits.

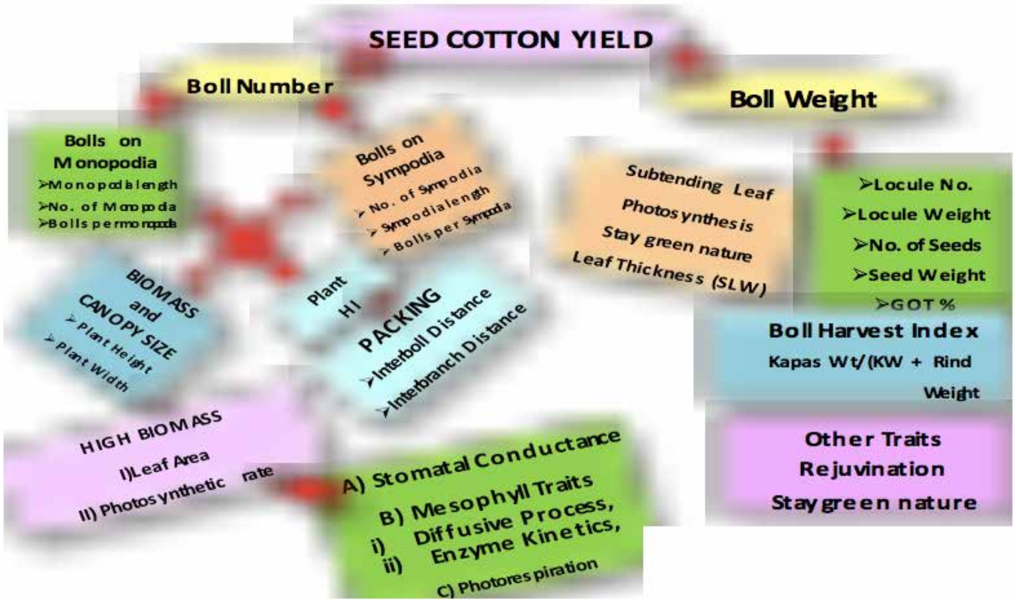


Figure 1. Path of productivity in cotton

Efficiency of releasing useful variability improves when multiple parents accounting for the desirable expression for an array of characters are used as they account for better complementation of an array of important traits as compared with the use of just two parents.

We studied variability in different combinations of four carefully chosen diverse parents representing genetically diverse (heterotic) groups to compare with that based on two parents. For this purpose, four parents were used from proven plant type diversity groups such as robust, stay green types on one side and compact, high relative growth rate (RGR) types on other side. It was observed that the proportion of transgressive segregants was much higher when such four-parent based segregating populations were developed for practicing selection in segregating generations (Edke, 2016). These results clearly indicate that for effective varietal improvement (genetic gain) in cotton, the existence of diversity of parents and complementation for the entire range of traits is very essential.

Effective modifications in approaches of handling segregating populations

After creation of useful variability, the next dimension of factors operates at exploitation of the variability created through hybridisation. For this, it is essential to recollect some basic concepts related to the consequences of selfing.

Consequences of selfing and constitution of selfed generations

Simple Mendelian expectations of segregation at a locus form the basis for determining the constitution of plants in the segregating populations derived through selfing. A heterozygous F_1 segregates to give 2^n gametic types and 3^n genotypic classes. As the number of loci influencing the ultimate dependent character like yield goes on increasing, values of total number of genotypes produced in F_2 generation reaches astounding figures. Breeders should be able to distinguish between a minimum population size and an optimum population size to be raised in the F_2 generation. It is interesting to know how this minimum expected population size changes over different segregating generations. When an F_1 segregates for a meagre 21 loci over 2 million gametic types are produced by F_1, and when these gametes unite, 10 billion genotypes are produced and this demands a minimum population of 4 trillion plants raised in F_2 generation and this is far less than the optimum population size required to be raised for these many loci. Though Allard (1960) addresses the issue of minimum population size, no mention is made about the optimum population size required to be grown. It is difficult to achieve optimum plant population size even when a F_2 population is segregating even for 21 loci? If a realistic number of loci (hundreds) governing inheritance of a dependent character like yield are considered, the genotypic classes and minimum population size reach astronomical figures and the population size normally used for handling F_2 segregating generation becomes abysmally insufficient to capture the variability. If the entire range of variability is not captured, it is difficult to expect the most potential genotypes to be seen in small population raised by a breeder. The cartoon (Fig. 2) highlights how small population size becomes just incompetent to capture the full range of variability released in F_2 generation obtained from a cross of truly diverse parents (segregating for hundreds of loci).

Figure 2. Cartoon depicting contradictions about optimum plant population and actual plant population on which a breeder exercise selectionwith an aim to develop most ideal variety.

National programmes consisting of team of breeders and supporting scientists

Many of the problems of cotton are too complex to be tackled by individual breeder and therefore there is a dire need for national level programs aimed at concerted efforts of team of breeders across different centres and supported by scientists of other related disciplines like entomology, pathology, physiology, agronomy, etc., so that the seeds of the broad-based populations developed in this manner are distributed to individual breeders for selection and stabilisation.

Such programs can generate useful data. One of the objectives of research could be to determine what happens when the F_2 population is shared and distributed to number of breeders at various locations so that its true potentiality is realised somewhere by some breeder compared to the results obtained by a single breeder — who would have created a huge useful variability but ultimately would have struggled to handle the transgressive segregants. There is a need for developing a yardstick for determination of the acceptable optimum population size based on the visible diversity among the parents for different yield related traits. It is essential that breeders exercise modifications in procedures of handling segregating generations along with a team of subject experts like entomologists, agronomists, pathologists for help in determining the target genotype and to ensure higher success rate.

Thus far, there are hardly any studies conducted to determine the minimum F_2 population of cotton that should be raised and to evaluate the possible impact of a small sample size (a few hundred plants) on the reduction in observed variability and loss of transgressive segregants. We compared the variability and frequency of transgressive segregants out of 2000 plants by taking a random sample of 200 plants each time out of this reasonable F_2 population for recording observations on productivity traits. Our data show how the most potential plants are often missed when a small population sample is examined. If the cross is segregating for realistic number of loci — hundreds, for example — it is impossible to capture the essence of the total variability released by the cross.

As an outcome of this study, it is suggested that whenever diverse parents are used in hybridisation and large amount of variability is released, individual breeders will not be able to handle such a large F2 population for capturing the most potential transgressive segregants. Instead, if such plans are executed through national programmes and the segregating population can be shared to groups of breeders working at different locations. This will potentially increase the chance of identifying the most potential segregants which can later be shared with the breeders of the region. In this manner, such segregating populations of planned diverse crosses can be exploited better by a team of breeders.

Innovative steps to recover the most potential transgressive segregants

The genetic explanation amply reinforces the fact that just as the blue whale is missing in the bucket of the breeder, the most potential transgressive segregants are missed from the small F_2 population. Now the condition of the cotton breeder is comparable to the fate of the passenger who reaches the railway station with heavy luggage just to discover that the train has already left the station. The challenge of catching the missed train could be compared to the challenge of creating the most potential transgressive segregants.

If the F_2 population is sufficiently large — thousands — the visible most productive segregants carry many of the desirable alleles distributed among them. This is comparable to a situation when an ardent fan of a famous celebrity is ridiculed by tearing the photo of the celebrity star into pieces (Fig. 3) and the fan out of dedication collects these pieces to recreate the lost picture to the extent possible. Only when the productive segregants are recombined, it is possible to recreate most potential segregants which were

Figure 3. Recombination of characters in F_2 populations and their recreation by intermating promising F_2 plants.

missed in segregating generation because of small population size raised.

In a study conducted at Dharwad on a segregating F_2 generation of *G. hirsutum*_varietal cross, the top 20 plants were advanced to F_3 in the off-season and were recombined through simulated random mating. The recombined population resulting from recombining the genetic backgrounds of these progenies was evaluated along with the normal selected plants progenies of the cross and normal (unselected) population. The mean, variance and frequency of transgressive segregants observed in this churned (recombined) population was found to be improved over the population derived by just selecting for high yield and the random population. This highlights the need for a mandatory step of recombination among the available potential segregants in early segregating generations to generate the missing most potential transgressive segregants.

Modifications in the procedures of handling segregating generations of cotton crosses

Depending on the extent of genetic divergence encountered by the breeder, it is possible to plan modifications in procedures for creation of variability and to exploit the same through selection by appropriate handling of segregating populations. Some of the modified procedures are provided below along with their genetic basis.

Genetic basis of some modifications

By F_6 generation, the minimal population size reduces from 4^n to 2^n per locus which gives a huge advantage for a large scale of segregation. Therefore, a method such as single seed descent (SSD) gets a blanket benefit over pedigree/bulk methods of breeding. There is a need to compare the efficiency of SSD over other methods in terms of reduction in minimum population size. It is suggested to delay the selection up to F_3 generation and F_2 population can be forwarded to F_3 generation exploring the option of off-season, wherever available. Postponing the artificial selection just by one generation (to F_3) provides a big advantage, considering the large number of genes for which the population is segregating as, the minimum population to be evaluated gets reduced to 3^n per locus.

Combination of SSD and pedigree methods of breeding

The main advantage of SSD is the reduction in population size to be evaluated for selection. When segregating generations are handled, minimum population size reduces from 4^n in F_2 to 2^n by about F_6 and later. This opens up possibilities of series of modifications of handling selfed segregating generations. Even if selection is postponed to F_3 by raising F_2 in off season through close spacing or broadcasting method of sowing and advancing F_2 to F_3 the minimum population size reduces approximately to 3^n in F_3. Intermating among the selected promising F_3 plants may be attempted to recreate the potential genetic variability which could have been achieved by evaluating large F_2 population. This greatly increases the chance of obtaining the most potential transgressive segregants. By following this deviation, it is possible to save one season and even gain a big advantage of reducing minimum population size.

Exploring the nature of gene action for higher genetic gains

It is possible to exploit both additive and complete dominance gene action in often-self-pollinated crops like cotton. To exploit dominance gene action, it is necessary to have lesser heterozygosity in plants to be selected. Hence, it is desirable to avoid selection in early generation like F_2 to F_4. By doing so, the deceptive level of genotypes in general reduces. This becomes an additional advantage apart from the advantage of reduced minimum population size.

Initial generations through SSD

The initial two segregating generations (F_2 and F_3) resulting from selfing show higher rise in inbreeding coefficient (to 0.75) thereby indicating that it is beneficial to follow SSD in these two early generations by raising very large population since the aim is to get just one seed from each segregant in these F_2 and F_3 generations. Artificial selection can very well be initiated in F_4 generation and all the benefits of reducing minimum size and associated benefits of starting artificial selection in F_4 can be exploited.

Alternate generations through SSD

Another alternate procedure is to grow even numbered segregating generations in off season and odd numbered segregating generations in the regular season. These procedures also ensure quickest advancing to later generations and exploiting the benefit of both pedigree and bulk method of breeding. Here, it is necessary that during off-season while advancing the population, identity is maintained for each plant by proper labelling. The F_2 population, in particular, needs to be raised during the off-season as the inbreeding coefficient increases rapidly.

Representing two generations in an evaluation

Using seeds of plants in regular season and off-season of each year, one can explore evaluation of two generations in a single row (season). Here the seeds of earlier generation (say F_3) may be used for evaluation while, at the end of each row, a small extended row (corresponding progeny of F_3) may be planted (Fig. 4). Based on performance, superior F_3 row lines can be selected but corresponding F_4 row may be harvested for advancing it to F_5 for evaluation. One can also explore to evaluate both F_4 and F_5 rows of selected lines in next year as this will help in confirming the performance of the selected lines with two generations grown in a single season per year. The suggested modifications have general value in all self-pollinated crops and cotton breeders should make use of these innovative modifications by exercising care in handling segregating generations.

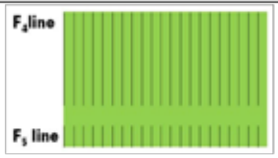
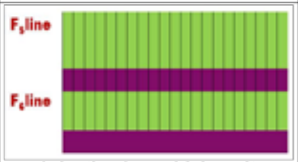
Year	Regular season	Off-season
2015	Evaluation of F_1 generation	Broadcast F_2 seeds to raise large population
2016	Evaluation of F_3 generation	F_4 generation through SSD
2017		F_6 advanced from selected F_5 lines identified based on the performance of F_4 lines
2018	 *Selection for multiple traits*	With every passing year, minimum population to be grown for selection of most productive segregants decreases significantly

Figure. 4. Evaluation of two generations in the same season for improving selection efficiency

Genetic basis of backcrossing

We uncovered many interesting facts upon our detailed studies on the constitution of segregating populations as compared to constitution of backcross derived populations. The proportion of homozygous genotypes is equal to proportion of plants resembling recurrent parent. Hence, the proportion of plants which are exactly like recurrent parent is still very less in even BC_5 generation. It is the proportion of plants nearly like recurrent parent that becomes more than 90% of the BC_1 population.

By deciphering similarities and differences in consequences of selfing and backcrossing, it is possible to show that the size of backcross populations normally involved in backcrossing is very small and this can adversely affect the recovery of constitution of backcross derived generations. The minimum population size is 2^n in BC_1 generation. It works out to be 2 million plants if the genetic background is definable by just 21 genes and considering a large genetic background (n in hundreds), this becomes a very large population size. As against this requirement, the commonly used size of a BC_1 population for backcrossing is much smaller. Due to sampling effect, it can lead to drift in allelic frequencies which may slow down the recovery of constitution of the recurrent parent. The implications of these findings are that the number of plants carrying the trait under transfer from donor parent have to be large and indeed this becomes the BC population which must account for the entire range of variability released in the population. During regular backcross breeding programs of cotton meant either for transferring Bt genes or for other traits, whenever very few plants are used in different backcross generations, the introgressed or improved version show differences from the original version of recurrent parent used in backcrossing. It should be noted that the effect mentioned here is specifically for the background genes (loci) of the genotypic constitution which are not linked to the gene under transfer. The problem of delay caused in recovery of constitution of recurrent parent adds another dimension to the problem. As an implication of this, it is necessary for the breeder to use a large number of plants and even observe progeny row from each plant used for back crossing for its resemblance with the recurrent parent and preserve remnant backcross and selfed seeds of each plant for a possible future use.

Conceptualisation of *'target genotype'*

When hybridisation work involving the chosen parents is initiated, the breeder should have a clear perception of the constitution of the most potential transgressive segregant (aimed to become constitution of the new variety). The idea is to define such a genotype as *'target genotype'* in terms of the proportion of alleles retained from the two parents chosen for hybridisation (it can be defined in terms of multiple parents as well but for simplicity sake, the case of using only two parents in hybridisation is considered). The perception of a target genotype should be clear to the breeder and this enables the breeder to choose an appropriate breeding method and even to make suitable modifications in handling the segregating generations.

Procedures of handling segregating generations after hybridisation can be broadly grouped into a) Pedigree/ Bulk/ SSD method and b) Backcross method of breeding. This distinction between the breeding methods can be made in terms of the proportion of alleles from two parents (involved in crossing) observed among the segregants in the generations derived (after hybridisation) in these methods of breeding. Backcross method of breeding is used when the donor parent has highly undesirable genetic background except for one desirable simply inherited trait (Allard, 1960). Here, the proportion of alleles required from the two parents in the target genotype would be defined as 98:2 or 99:1. The genetic consequences of backcrossing and about when to use backcross breeding are well explained in different books on plant breeding and the procedure followed during backcross breeding precisely increases the chance of occurrence of such a target genotype.

The backcross method of breeding is considered as uniquely scientific especially because this breeding procedure facilitates and enhances the chance of occurrence of the target genotype (something like 99:1 in terms of proportions of alleles from the two parents) by modifying constitution of the base F_1 population (with 50:50 allelic proportions of the two parents) developed through hybridisation. In every backcross generation, the proportion

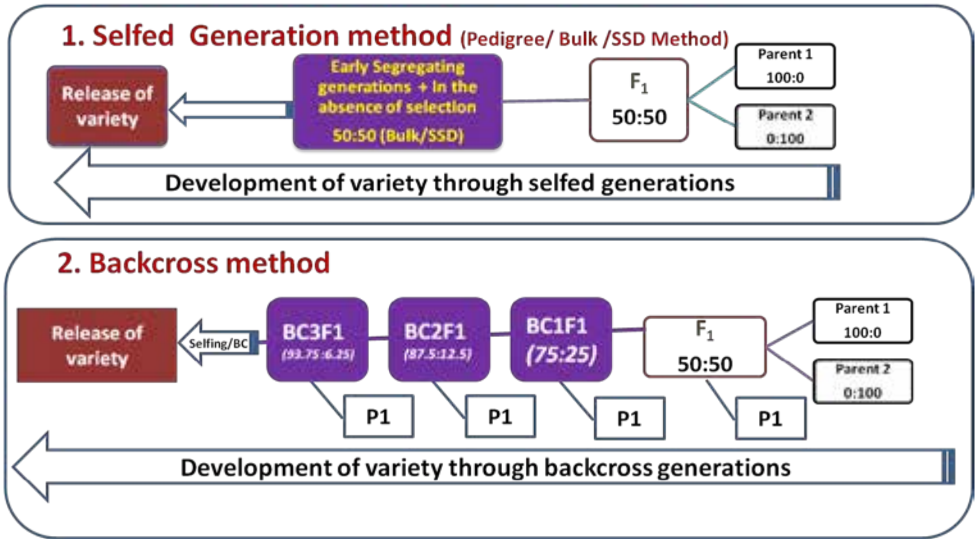


Figure 5. Handling the material after hybridisation in varietal breeding and the proportion of alleles from parents

of alleles from the recurrent parent goes on increasing and the undesirable alleles of donor parent are flushed out at high speed. If the breeder keeps track of the desirable trait of the donor parent under transfer and holds that intact in the plants chosen for back crossing, the targeted genotype is produced with great ease.

Varietal development vis-à-vis proportion of alleles from parents

With breeding methods such as Pedigree, Bulk and SSD, is it possible to expect a high frequency of target genotype? How do they compare with backcross breeding method in terms of efficiency in generating high frequency of the desired target genotype? Should these methods be rated as less scientific because the breeder is not clear about the expected target genotype set and hence thinks that it is not achieved? These are the important aspects on which a lot of information has to be generated through breeding system research, but there is a dearth of planned research in this direction. In the entire population of plants in a segregating generation, the average proportion of alleles of the two parents in F_2 and any subsequent segregating selfed generation is 50:50. It has been worked out and shown (Patil, 2014) that when genotype of each plant is examined in terms of alleles contributed by the two parents and the frequency distribution is worked out, it is observed that the proportion of plants with allelic contribution being equal to or close to 50:50 is highest (Table 1).

When this explanation is extended to cover polygenic traits, the distribution assumes the shape of normal curve. The inference derived from this distribution is that all three procedures of handling segregating generations (pedigree, bulk and SSD) will be successful when the desirable alleles for all the loci are distributed equally among the parents used for hybridisation. It also means that if the distribution of desired alleles is uneven, the pedigree method of breeding will not be successful. In contrast to this, in backcross breeding, the proportion of alleles from recurrent parent goes on increasing with every backcross generation. This is always seen as a major difference in the consequence of selfing and backcrossing. The very purpose of back crossing is to decrease the proportion of alleles of the donor parent which are associated with an undesirable genetic background. With continuous backcrossing, there is a methodical and rapid increase in the proportion of plants (in the backcross population) which resemble the recurrent parent.

The segregants most commonly observed in a breeding approach should match with the target genotype conceptualised in the beginning of varietal improvement program. Otherwise, the selected selection approach may not give the desired results. Hence, it is necessary to understand the kind of target genotype set by the particular pair of parents before embarking on the choice of breeding procedure. It is also equally important to understand the types of segregants most commonly observed in a selected breeding approach.

A segregation ratio of 1:2:1 is observed in F_2 generation with respect to a locus under consideration. When segregation at two loci is considered, a ratio of 1:4:6:4:1 is observed where the segregants with 50:50 allelic contributions from the two parents are most common (Table 1). The extreme types and those with unequal contribution of alleles from the two parents are less common. Extending it to three loci, a ratio of 1:6:15:20:15:6:1 is seen in F_2 and here again segregants with 50:50 or nearly 50:50 allelic contribution from the two parents occur most commonly in an early segregating generation. This pattern of segregation remains the same even when a large number of loci affecting a quantitative character is considered as depicted in Figure 6 and 7. In both methods of Bulk and SSD, artificial selection begins at a stage when the population is fixed or nearly fixed at the segregating loci governing inheritance of quantitative traits and at this stage the number of gametic classes and genotypic classes are same(2^n). With the help of Pascal's triangle, the segregation ratios can be worked out in F_2 generation and also in the generation representing fixation.

Table 1: Proportion of alleles of two parents among segregation ratios

a) F_2 generation

Genotypes and proportion of alleles of two parents

One gene case	AA	Aa	Aa
Proportion of alleles of parents	100:00:00	50:50:00	0,06944444
Segregation ratio	1	2	1

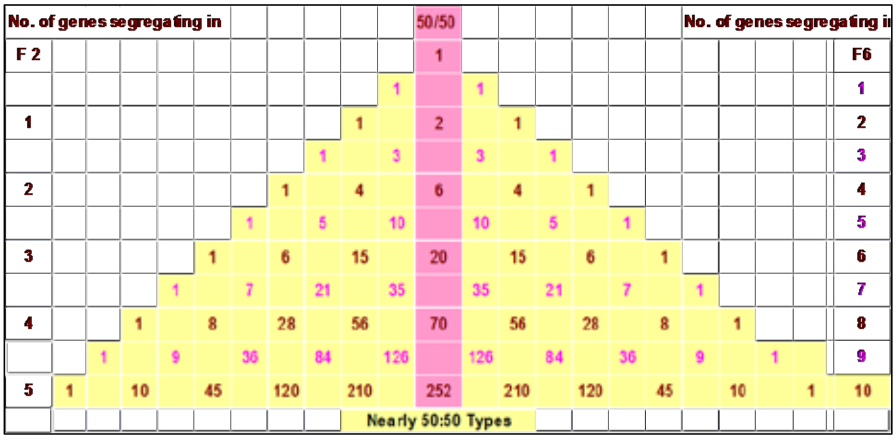
Genotypes and proportion of alleles of two parents

Two gene case	AABB (1)	AABb (2) AaBB (2)	AaBb (4) AAbb (1) aaBB (1)	aaBb (2) Aabb (2)	aabb (1)
Proportion of alleles of parents	100:00:00	75:25:00	50:50:00	25:75	0,06944444
Segregation ratio	1	4	6	4	1

b) F_6 generation

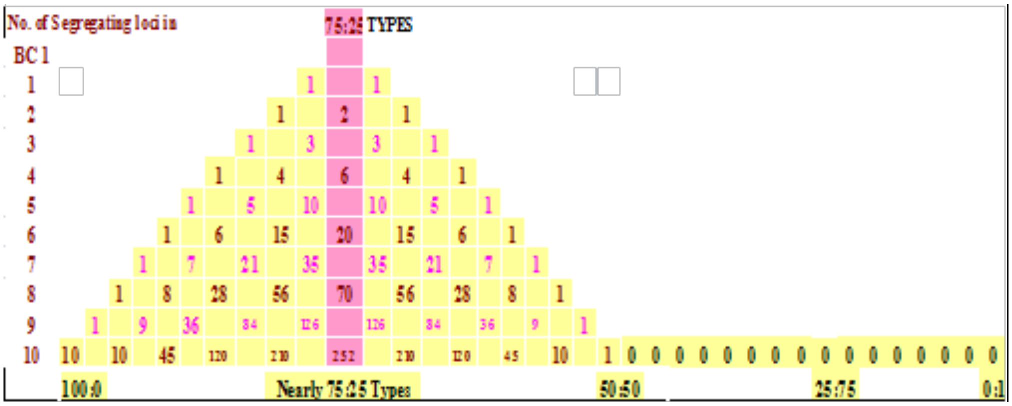
Genotypes and proportion of alleles of two parents

Two gene case	AABB (1)	AAbb (1) aaBB (1)	Aabb (1)
Proportion of alleles of parents	100:00:00	50:50:00	0,06944444
Segregation ratio	1	2	1



No. of genes segregating in											50/50											No. of genes segregating i
F 2											1											F6
										1		1										1
1									1		2		1									2
								1		3		3		1								3
2							1		4		6		4		1							4
						1		5		10		10		5		1						5
3					1		6		15		20		15		6		1					6
				1		7		21		35		35		21		7		1				7
4			1		8		28		56		70		56		28		8		1			8
		1		9		36		84		126		126		84		36		9		1		9
5	1		10		45		120		210		252		210		120		45		10		1	10
											Nearly 50:50 Types											

Figure 6. Proportion of alleles from the two parents among segregants in early as well as later segregating generations



No. of Segregating loci in											75:25	TYPES																									
BC 1																																					
1										1		1																									
2									1		2		1																								
3								1		3		3		1																							
4							1		4		6		4		1																						
5						1		5		10		10		5		1																					
6					1		6		15		20		15		6		1																				
7				1		7		21		35		35		21		7		1																			
8			1		8		28		56		70		56		28		8		1																		
9		1		9		36		84		126		126		84		36		9		1																	
10	10		10		45		120		210		252		210		120		45		10		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	100:0										Nearly 75:25 Types										50:50									25:75							0:1

Figure 7. Proportion of alleles from the two parents among segregants in BC1 generation

In Figure 6, it is observed that the trend of prominence of 50:50 types among segregants continues even at this stage of fixation. It means that whether it is pedigree or bulk or SSD method of handling segregating populations, 50:50 types of segregants are most commonly seen in these populations subjected to artificial selection. As compared to this there is a lower frequency of 70:30 types (or symmetrically 30:70 types) and an even lesser frequency of 95:5 (or 5:95) type. It is important to remember for this reason that the target genotype achieved through back cross method of breeding (say 98:2 type) is very much available even in F_2 generation but locating such segregants in F_2 becomes a Herculean task. Therefore, it is avoided, and the tedious procedure of continuous back crossing is preferred over selection for the target 98:2 type in F_2 or later segregating generation.

Thus, the selection processes practiced in these segregating populations will be successful only if the 'target genotype' set by the pair of hybridised parents is close to 50:50. This means that if the two parents are perfectly complimenting each other on gene for gene basis or crudely speaking trait for trait (component trait) basis, the target genotype set will be 50:50 type. In simpler terms, in any segregating generation (F_2, ... F_6.), the segregants which are 50:50 types (50% alleles from each parents) are the most commonly observed types. If the two parents are complementing each other perfectly by sharing the desirable alleles each at 50% of the total number of loci responsible for yield, then the target genotype in case of the parental pair chosen for hybridisation is a 50:50 type. Since the segregating population consists of mainly 50:50 types the task of the breeder is restricted to selecting the *"Best 50:50 type"*, which could be considered as the target recombinant type. This incidentally it is a positive transgressive segregant which blends the desirable favourable alleles equally distributed between the two parents. Apart from these extreme positive transgressive segregants among this wide array of 50:50 types of segregants, one can also expect extreme negative transgressive segregants, which may perfectly blend only the undesirable alleles distributed equally from the two parents. Since the population has high frequency of 50:50 types, selecting the best 50:50 among them becomes relatively easy. Based on this theory, it is expected that these three methods of handling segregating populations after hybridisation will be successful only when the target genotype is a 50:50 type. It should also be remembered that following these methods will be ineffective if the parents used in hybridisation possess unequal distribution of desirable alleles (traits) between the parents (say 70:30 or 80:20). It is because the frequency of the desirable genotypes in early or later segregating generations is very low. Searching for the best 80:20 in a population containing a full of an array of 50:50 types at a very low frequency occurrence of these different 80:20 types is perhaps equivalent to searching a pin in a haystack. Very often plant breeders fail to find potential transgressive segregants in the segregating generations and it is therefore important to understand reasons for the failures which often depends on how the segregating populations are handled during selection and stabilisation process.

The methods of handling segregating generations are well defined when the target genotype is 50:50 type (Pedigree/ Bulk/ Single seed descent method) or an extreme type such as 99:1 or 98:2 or 100:0 (Backcross breeding). It is advised to practice limited backcross breeding when the target genotype is in between these two types, say 70:30 or 80:30. In a single backcross derived populations (BC_1F_2, BC_1F_3... etc.,), 75:25 types are most commonly observed and hence it is easier to expect higher frequency of 70:30, 75:25 or 80:20 types (Fig. 7). Here again, the task of the breeder is simplified to the extent of finding which 75 are seen from the first parent and which 25 are seen from second parent. When majority of segregants are 75:25 types, it becomes easy to pick up the extreme positive transgressive segregants with the required desirable 75 alleles from the first parent and 25 from the second parent.

Thus, selection in a limited backcross-derived population (single backcross) would be successful when the desirable alleles are unequally distributed between the parents (say around 75:25) while selfed breeding methods will be more efficient when parents possess nearly equal distribution of desired alleles between them. The utility of limited backcross approach of breeding has been highlighted earlier by Patil (2007, 2011).

Backcross derived Pedigree/Bulk/Single seed descent method

It is important to note that limited backcrossing just refers to creation of base BC_1F_2 population with higher frequency of the target genotype. Once such base population is created, the breeder has many options of initiating artificial selection right from the BC_1F_2 generation. This modified approach is similar to pedigree method of breeding and can be continued by implementing selection schemes described for F_3, F_4 etc., in the corresponding BC_1F_2, BC_1F_3 etc. generations, respectively. If the considerable heterozygosity existing in a backcross derived population is considered as a disadvantage, then the artificial selection can be delayed up to BC_1F_5 when the proportion of homozygous plants is increased substantially. Either bulk method of advancing or SSD approach can be followed through these early segregating generations. There is a need for research on comparing efficiency of following Pedigree/SSD/Bulk methods in handling segregating generations derived through limited backcross breeding

Following the approach of determining the target genotype involves comparison of backcross Populations to P_1 (BC_1) and P_2 (BC_2) with F_2 population. The segregation pattern observed in BC_1, BC_2 and F_2 populations shows differences in prominent types namely 75:25, 50:50 and 25:75 types in them. This becomes the genetic basis for understanding the differences in means of these populations. If the target genotype set by the pair of parents involved in hybridisation matches with the prominent segregant type observed in a generation, the mean performance of that population will be higher than the mean of the remaining two populations. As per this basis of inheritance, any of the following three situations can be observed in an evaluation study where these three segregating populations are compared.

Table 2: Comparison of Means of Segregating Populations

Situation I: *Decreasing order of performance being $BC_1 > F_2 > BC_2$*

Population	Highest Proportion of alleles of parents		Method of breeding	Result of selection in the population
	P_1	P_2		
BC_1 (F_1 X P_1)	75	25	Limited backcross breeding (with P_1)	More rewarding
F_2 (Selfing F_1)	50	50	Pedigree/Bulk/SSD	Less rewarding
BC_2 (F_1 X P_2)	25	75	Limited backcross breeding (with P_2)	Least rewarding

Here the P_1 parent has higher proportion of desired alleles contributing to higher productivity, and as a result of this, the decreasing order of performance of populations will be $BC_1 > F_2 > BC_2$. In such a case, selfed generations of BC_1 populations can be subjected to selection either by following pedigree or bulk or single seed decent methods of breeding.

***Situation 2:** Decreasing order of performance being BC_2 >F_2 >BC_1*

Population	Highest Proportion of alleles of parents		Method of breeding	Result of selection in population
	P_1	P_2		
BC_1 (F_1 X P_1)	75	25	Limited backcross breeding (with P1)	Least rewarding
F_2	50	50	Pedigree/Bulk/SSD	Less rewarding
BC_2 (F_1 X P_2)	25	75	Limited backcross breeding (with P2)	More rewarding

Here the P_2 parent has higher proportion of desired alleles contributing to higher productivity because of which these populations reveal a decreasing order of performance of BC_1>F_2>BC_2. In this situation, selfed generation of BC_2 populations can be subjected to selection either by following pedigree or bulk or single seed decent method of breeding.

***Situation 3:** Decreasing order of performance being F_2 >BC_1 or BC_2.*

Population	Highest Proportion of alleles of parents		Result of selection in population
	P_1	P_2	
F_2	50	50	More rewarding
BC_1	75	25	Less rewarding
BC_2	25	75	

Here P1 and P2 parents have an equal proportion of desired alleles contributing to higher productivity of F2>BC1>BC2 or F2>BC2>BC1. In this situation advancing selfed F2 and later segregating generations can be subjected to selection either by following pedigree or bulk or SSD method of breeding.

Studies on determining the 'target genotype'

To test this concept, a study was conducted to understand the differences in performance of the three segregating populations and their subsequent selfed generations in cotton at Dharwad, India. Among the three crosses handled in RAH100 X SAM 4, BC_1 population revealed higher mean seed cotton yield/plant and this trend of superiority was continued in the next selfed generation, confirming that the trend of observed superiority occurring in this population did continue in the next selfed generation (Table 3). Many transgressive segregants were noticed in this population. As per the expectations of the genetic basis explained earlier, RAH 100 has many important yield contributing characters including boll number because of which BC_1 and the subsequent selfed population revealed improved performance as compared to the remaining two populations. In a cross of DSC 7 X RAH 53 involving a compact parent and a stay green type, F_2 population was significantly superior to B_2 indicating that the parents were complimenting each other for the yield influencing loci due to which F_2 revealed superior performance and this trend of superiority continued in F_3 generation. In RAH 111 X RAH 16, BC_2 population revealed higher population mean as compared to the other two populations and this trend continued in next selfed generation. The number of transgressive segregants observed in these populations in general matched with the higher mean seen in the populations. Thus, different combinations of parents included in the study differed with respect to the pattern of complementation of genes for which they differ.

The parental genotypes used in the study were evaluated for an array of yield attributing characters including physiological characters related to photosynthesis. An overall assessment of parents for these many important traits gave insight on complementation pattern of desirable traits (alleles) between the elite genotypes chosen. This assessment matched with the previously mentioned conclusion derived from comparing the three segregating generations — F_2, BC_1 and BC_2.

In another study, different populations with gradation of allelic contributions from the two parents were developed. This study involved two intra-*hirsutum* crosses involving four varietal lines 1) DRGR 2572 x M 5 and 2) DRCR 4 X DSMR 10. The nine generations (P_1, P_2, F_1, BC_1, BC_2, F_2, BC_1F_2, BC_2F_2, F_3) of both crosses were sown and evaluated in kharif 2015. The data on these generations confirmed that the trend of superior performance and gradations in performance is confirmed in succeeding selfed generations. Further in an effort to exploit the variability released in these populations top 30% of the best plants were selected from the BC_1, BC_2, F_2, BC_1F_2, BC_2F_2, F_3 generations and All the nine generations (P_1, P_2, F_2, BC_1F_2, BC_2F_2, F_3, BC_1F_3, BC_2F_3, F_4,) of both crosses

Table 3. Comparison of Performance of Limited Backcross and Selfed Populations for Seed Cotton Yield (g/plant)

Cross	2007-2008			2009-2010		
I. RAH100 X SAM4	BC_1	F_2	BC_2	BC_1F_2	F_3	BC_2F_2
No. of plants/lines (L)	154	468	198	96 L	287 L	71 L
Mean Seed Cotton Yield(g/pl)	167.1*	144.3	129.8	138.6*	118.5	99.4
II. DSC 7X RAH 53						
No. of plants/lines (L)	166	398	184	78 L	243 L	97 L
Mean Seed Cotton Yield(g/pl)	174.8	189.1*	131.7	142.8	154.9*	139.6
III. RAH 111 X RAH 16						
No. of plants/lines (L)	165	371	200	77 L	216 L	90 L
Mean Seed Cotton Yield(g/pl)	157.7	150.4	169.0	121.4	146.2	164.1*

*Significantly higher.

were sown for evaluation in kharif 2016 (Adarsha, 2017). The superior variability observed in the specific populations responded to selection practiced for high yield. This confirms that the higher mean and variability seen in back cross populations responds to selection and confirms that these modified procedures are effective in achieving higher genetic gain for productivity enhancement.

Genetic basis of exploiting heterotic groups and improving performance of hybrids

Performance of hybrids depends upon genetic diversity and complementation of parents for loci influencing different important traits between parents. In a self-pollinated crop like cotton, in which inbreeding depression is not much of a concern, it is possible to observe *per se* performance by grouping genotypes into heterotic groups before understanding their combining ability pattern. Continuous efforts made on relating hybrid performance in cotton with genetic diversity existing among parents has led to development of heterotic groups in cotton. In general, crosses between robust, bushy types and compact types were found to be heterotic because of complementation between these groups. In addition, the stay green groups of genotypes combined well with high 'relative growth rate' types and also compact types. Several studies were conducted to identify elite combiners of each group and exploit these heterotic groups for improving combining ability by practicing procedures such as reciprocal selection for combining ability (Patil et al 2007, Patil and Patil 2007, Patil et al., 2011, Pranesh and Patil 2014). Initially, variability for favourable dominant alleles was created by using two parents in each group. In recent years, broad based populations are developed based on four parents in each group. Other studies on checking consistency of combining ability over generations was carried out. Though potential crosses were obtained between F_3 lines and the opposite groups testers, performance of crosses between the new double cross F_3 lines of the opposite groups were found to be of more potential. This indicates diversity created between the lines of opposite groups obtained from four parent crosses. Development of populations of opposite groups based on four elite combiner parents appears to be a promising approach in exploiting heterotic groups.

Two different breeders with varied understanding of genetic principles can produce different results even after handling the same population for developing a variety or the same germplasm for developing a hybrid. The innovative approaches followed in the creation of useful variability required for varietal development and modifications in approaches of exploiting this variability have shown encouraging results indicating in general that instead of the conventional varietal development approaches there is a need to incorporate these innovative modifications in breeding methodologies for developing superior varieties and hybrids.

Developing broad-based populations under national programmes

There is a need to develop broad based populations by pooling genes of resistance to diseases like CLCuV, and pests such as jassids and bollworms, which are known for their ability to quickly develop resistance to protection measures used against them. Plant breeding solutions of incorporating such special traits should involve pooling genes for resistance by utilising resistant sources. In addition to wild species, diploid species can also be explored for improvement of these traits. The approaches of introgression through systematically planned, limited backcrossing may become necessary. The size of a backcross population grown/found to be resistant/and used for backcrossing needs to be distinguished. The optimum/minimum population sizes assume astronomical figures and the implications of a limited sample size on the chance of recovering 'recurrent parents constitution' becomes more difficult than what is assumed in consequences of backcrossing.

National/continental programs of exploring useful alleles in wild species through limited introgression

Wild species need to be explored for the hidden valuable genes, alleles for stress tolerance and rare alleles associated with the main productivity traits. It is essential to initiate national programs of developing backcross programs involving wild species and diploid species. While practicing backcrossing, it is essential to understand and distinguish concepts of minimum and optimum population sizes to be grown with plants carrying the desirable target trait and their influence on recovering constitution of the recurrent parent.

Use of induced mutations to supplement and compliment recombinational variability

It is possible to complement hybridisation with induced mutations to generate more useful variability. In addition, induced variability can be helpful in producing rare alleles for biotic and abiotic stress resistance. Apart from inducing mutations for creation of variability in varietal breeding, it can be used to create variability for gene action and multiple alleles helpful in hybrid breeding.

Development of heterotic populations for common use by breeders

The modified procedures of 'reciprocal recurrent' selection which are basically defined for cross pollinated crops are aimed at increasing genetic diversity between opposite populations. The phenomenon of heterosis is basically having the same genetic basis of $H_{F1} = \sum dy^2$ and implications of the formula clearly show that by increasing genetic diversity, it is possible to increase the magnitude of heterosis. A series of these studies on exploiting heterotic groups have proved that it is possible to transcend the limits of mating system and utilise the procedures originally described for cross-pollinated crops used for improving self-pollinated crops as well.

Options of breeding genetically modified (GM) events

Populations or pools of alleles should be built with active and effective GM events. Apart from deregulated events like Mon531, some new events are being compared and many more are being developed and identified. New events from UAS, Dharwad (cry1Ac event, UASD No. 78), Delhi University (cry1Ac event, Tg2E13), NBRI, Lucknow (Tma12 & Cry1Ec events) are prominent among them. There is a need for identifying the most effective ones among these events to incorporate them into elite varieties and release them for commercial cultivation through fast-track approaches. With appropriate pyramiding or stacking of potential events, it is possible to achieve sustainable cotton production in the years to come. Providing access to these events for the breeders in state agricultural universities (SAUs) can expedite the progress.

Need for special support for conventional breeding research

There is a severe dearth of support for plant breeding research and procedures of varietal improvement and hybrid development. This paper draws the attention of cotton researchers to the recent findings from our plant breeding research and also toward some inferences and plant breeding implications derived from the basic principles of 'population and quantitative genetics'.

Programmes on varietal development and hybrid development that need to be approached by a team of breeders, covering different locations are highlighted above. Some additional joint ventures of teams of scientists which need to be supported with an open mindset are explained. Plant breeders often experience that that innovative ideas of research submitted as research projects generally do not attract the attention of funding agencies and this situation is highlighted in the cartoon (Figure 8). While molecular approaches can complement plant breeding approaches, there is a need for funding agencies as well as government institutions to appreciate the importance of innovative conventional breeding methodologies that may provide a much higher value of returns for investments than the investment made on molecular aspects for plant breeding.

References

Adarsha.,2017 Genetic studies on target genotype and response to selection in cotton. M.Sc(Agri) thesis submitted to University of Agricultural Sciences,Dharwad.p143

Allard R.W., 1960., Principles of Plant breeding, John Wiley and sons, Inc. New York, P.485

Patil, S. S. and Patil, S. A., 2003, Role of improving combining ability in increasing performance of cotton hybrids. Third World Cotton Res. Conference, Cape Town South Africa. 9-13 March 2003.

Patil, S. S., Patil, R. S., Deepak, C. A., Somashekhar, D. and Kolhar, B. C., 2003, Genetic evaluation and comparison of *G. herbaceum* and *G. hirsutum* varieties in different levels of rainfed and intensive management situation. Third World Cotton Res. Conference, 9-13 March, Cape Town, South Africa.,

Patil, S. S. and Patil, S. A., 2003, Use of plant type concept in Cotton breeding. Third World Cotton Res. Conference, Cape Town South Africa. 9-13 March 2003. P- 34.

Patil, S. S., Patil, S. A., Ramakrishna, V., Deepak, H. B. and Mallikarjun, H. B., 2007, Approaches of improving combining ability for enhancing performance of cotton hybrids. Proc Fourth World Cotton Research Conference held at Lubbock, USA, 1818.

Patil, S. S., 2007, Potential of limited backcross breeding in improving cotton. Proc Fourth World Cotton Research Conference held at Lubbock, USA, 1926.

Patil, S. S., Ramakrishna, V., Maralappanavar, M. S., Swathi, P., Ranganatha, H. M., Kencharaddi, H. G. and Babu, D. H., 2011, Deploying reciprocal selection for combining ability for improving performance of hybrids in cotton (*Gossypium hirsutum* L.). *Indian J. Genet.*, 71(2): 180-184.

Patil, S. S., 2011, Importance of teaching concepts of population genetics in effective understanding of plant breeding. *Indian J. Genet.*, 71(2): 106-114.

Patil, S. S., 2011, Target genotype based choice of breeding methods in cotton. World Cotton Research Conference – 5, November 5 – 10, Mumbai, India.

Pranesh, K. J. and Patil, S. S., 2014, Identification of transgressive segregants for combining ability in early generation of cotton. Green Farming, 5(5): 734-737.

Patil, S. S., 2014, Conventional breeding of cotton needs to change. ICAC Recorder, 3:13-20.

Vijayalaxmi, Udikeri, G., Narayanan, K., Nachne, R. P., Patil, S. S. and Kulkarni, V. N., 2011, Trends in cotton fibre quality improvement in India. *Cotton Res. J.,* 25: 84-92.

Vinayak Edke.,. 2016 Genetic studies on utilising heterotic groups for varietal improvement in cotton M.Sc(Agri) thesis submitted to University of Agricultural Sciences, Dharwad, p143.